

APPENDIX B

PENDING AND AMENDED CLAIMS

1. (Once amended) An isolated nucleic acid that encodes a fusion polypeptide, wherein the fusion polypeptide comprises:

a) a catalytic domain of a glycosyltransferase that catalyzes the transfer of a saccharide, from a saccharide donor comprising a nucleotide sugar, to an acceptor molecule; and

b) a catalytic domain of an accessory enzyme that catalyzes the formation of the nucleotide sugar.

2. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a eukaryotic glycosyltransferase.

3. (As filed) The nucleic acid of claim 1, wherein the accessory enzyme is a eukaryotic accessory enzyme.

5. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a prokaryotic glycosyltransferase.

6. (As filed) The nucleic acid of claim 1, wherein the accessory enzyme is a prokaryotic accessory enzyme.

7. (As filed) The nucleic acid of claim 1, wherein the fusion polypeptide further comprises a catalytic domain of a second accessory enzyme.

8. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is selected from the group consisting of sialyltransferases, N-

acetylglucosaminyltransferases, N-acetylgalactosaminyltransferases, fucosyltransferases, galactosyltransferases, glucosyltransferases, glucuronosyltransferases, xylosyltransferases, and mannosyltransferases.

9. (Once amended) The nucleic acid of claim 1, wherein the accessory enzyme is selected from the group consisting of:

- a GDP-mannose dehydratase;
- a GDP-mannose 3,5-epimerase;
- a GDP-mannose 4-reductase;
- a UDP-glucose 4' epimerase;
- a UDP-GalNAc 4' epimerase;
- a CMP-sialic acid synthetase;
- a neuraminic acid aldolase;
- an N-acetylglucosamine 2' epimerase;

a phosphate kinase selected from the group consisting of a pyruvate kinase, a myokinase, a creatine phosphate kinase, an acetyl phosphate kinase, and a polyphosphate kinase; and

a pyrophosphorylase selected from the group consisting of a UDP-Glc pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a GDP-mannose pyrophosphorylase, a GDP-fucose pyrophosphorylase, and a UDP-GlcNAc pyrophosphorylase.

10. (As filed) The nucleic acid of claim 1, wherein the nucleotide sugar is selected from the group consisting of GDP-Man, UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc, CMP-sialic acid, GDP-Fuc, and UDP-xylose.

11. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a sialyltransferase and the nucleotide sugar is CMP-sialic acid.

12. (As filed) The nucleic acid of claim 11, wherein the accessory enzyme is a CMP-sialic acid synthetase.

13. (As filed) The nucleic acid of claim 11, wherein the accessory enzyme is a neuraminic acid aldolase or an N-acetylglucosamine 2' epimerase.

14. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a galactosyltransferase and the nucleotide sugar is UDP-galactose.

15. (As filed) The nucleic acid of claim 14, wherein the accessory enzyme is a UDP-glucose 4' epimerase.

16. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a fucosyltransferase and the nucleotide sugar is GDP-fucose.

17. (As filed) The nucleic acid of claim 16, wherein the accessory enzyme is selected from the group consisting of a GDP-mannose dehydratase, a GDP-mannose 3,5-epimerase, a GDP-fucose pyrophosphorylase, and a GDP-mannose 4-reductase.

18. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is an N-acetylgalactosaminyltransferase and the nucleotide sugar is UDP-GalNAc.

19. (As filed) The nucleic acid of claim 18, wherein the accessory enzyme is a UDP-GalNAc 4' epimerase.

20. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is an N-acetylglucosaminyltransferase and the nucleotide sugar is UDP-GlcNAc.

21. (As filed) The nucleic acid of claim 20, wherein the accessory enzyme is a UDP-GalNAc 4' epimerase.

22. (As filed) The nucleic acid of claim 1, wherein the glycosyltransferase is a mannosyltransferase and the nucleotide sugar is GDP-Man.

23. (Once amended) The nucleic acid of claim 1, wherein the catalytic domain of the glycosyltransferase and the catalytic domain of the accessory enzyme are joined by a peptide linker.

24. (As filed) The nucleic acid of claim 1, wherein the nucleic acid further comprises a polynucleotide that encodes a signal sequence which is linked to the fusion polypeptide

25. (As filed) The nucleic acid of claim 1, wherein the nucleic acid further comprises a polynucleotide that encodes a molecular tag which is linked to the fusion polypeptide.

26. (Once amended) An expression vector which comprises the nucleic acid of claim 1.

27. (Once amended) A host cell which comprises the expression vector of claim 26.

33. (Once amended) A method of producing a fusion polypeptide, the method comprising:

- a) introducing into a host cell the expression vector of claim 26, under conditions where the host cell is transformed with the expression vector; and
- b) culturing the transformed host cell under conditions where the fusion polypeptide is expressed in the transformed host cell.

34. (Once amended) The method of claim 33 further comprising a step of purifying the expressed fusion polypeptide.

35. (Once amended) The method of claim 33 further comprising a step of permeabilizing the host cell expressing the fusion polypeptide.

36. (New) The nucleic acid of claim 1, wherein the accessory enzyme is a pyrophosphorylase.